PATENT
ATTY. DOCKET NO. REGEN1260-3

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicants:	Tsien et al.	Art Unit:	Unassigned
Application No.:	Unassigned	Examiner:	Unassigned
Filed:	January 25, 2002		
Title:	TANDEM FLUORESCENT PROTEIN CONSTRUCTS		

Box PATENT APPLICATION
Commissioner for Patents
Washington, DC 20231

Sir:

EV016236959US

PRELIMINARY AMENDMENT

In connection with the filing of the above-identified patent application, which is a Continuation of U.S. Serial No. 09/396,003, filed September 13, 1999, and prior to examination of the subject application, entry of the amendments and consideration of the following remarks respectfully are requested.

CERTIFICATION UNDER 37 CFR §1.10
"EXPRESS MAIL" Mailing Label Number: **EV 016 236 959 US**
Date of Deposit: **January 25, 2002**
I hereby certify that this correspondence is being deposited with the United States Postal Service as **"Express Mail Post Office to Addressee"** with sufficient postage on the date indicated above and is addressed to: Box PATENT APPLICATION, Commissioner for Patents, Washington, D C. 20231.
Aldon Griffis *(Name of Person Mailing Paper)*
Aldon Griffis *(Signature)* January 25, 2002 *(Date)*

I. AMENDMENTS

IN THE DRAWINGS

Please enter Substitute Figure 1B and Substitute Figure 2.

IN THE SPECIFICATION

Please delete the sentence at page 1, lines 3-4, and substitute therefor:

--This application is a continuation of U.S. Serial No. 09/396,003, filed September 13, 1999, which is a continuation of U.S. Serial No. 08/792,553, filed January 31, 1997 (now U.S. Patent No. 5,981,200), which is a continuation-in-part of U.S. Serial No. 08/594,575, filed January 31, 1996.--

IN THE CLAIMS

Please cancel claims 1 to 56.

Please add new claims 57 to 78 as follows:

--57. A tandem fluorescent protein construct, comprising a donor fluorescent protein moiety, an acceptor fluorescent protein moiety and a linker moiety that couples the donor and acceptor moieties and wherein the donor and acceptor moieties exhibit fluorescence resonance energy transfer ("FRET") when the donor moiety is excited by radiation, characterized in that the linker moiety comprises a protease cleavage recognition site, wherein cleavage of the linker by a protease results in a change in FRET between the donor and acceptor moieties,

and wherein the donor moiety comprises an *Aequorea* fluorescent protein (SEQ. ID. No. 2) comprising the amino acid substitutions,

a) Phe64Leu, Ser65Thr, Tyr66Trp, Asn146Ile, Met153Thr, Val163A and Asn212Lys, or

b) Ser65Gly, Val68Leu, Ser72Ala and Thr203Tyr, or

c) Tyr66His and Tyr145Phe, or

d) Tyr66Trp, Asn146Ile, Met153Thr, Val163Ala and Asn212Lys, or

e) Ser72Ala, Tyr145Phe and Thr203Ile, or

f) Ser65Thr, Ser72Ala, Asn149Lys, Met153Thr and Ile167Thr, and

the acceptor moiety comprises an *Aequorea* fluorescent protein (SEQ. ID. No. 2) comprising the amino acid substitutions,

a) Ser65Gly, Val68Leu, Ser72Ala and Thr203Tyr, or

b) Ser65Thr, Ser72Ala, Asn149Lys, Met153Thr and Ile167Thr.

58. A tandem fluorescent protein construct, comprising a donor fluorescent protein moiety, an acceptor fluorescent protein moiety and a linker moiety that couples the donor and acceptor moieties and wherein the donor and acceptor moieties exhibit fluorescence resonance energy transfer ("FRET") when the donor moiety is excited by radiation, characterized in that the linker moiety comprises a protease cleavage recognition site, wherein cleavage of the linker by a protease results in a change in FRET between the donor and acceptor moieties, and

wherein the donor moiety comprises an *Aequorea* fluorescent protein (SEQ. ID. No. 2) comprising the amino acid substitutions,

a) Tyr66His and Tyr145Phe, or

b) Tyr66Trp, Asn146Ile, Met153Thr, Val163Ala and Ans212Lys, and

the acceptor moiety comprises an *Aequorea* fluorescent protein (SEQ. ID. No. 2) comprising the amino acid substitutions,

a) Ser65Cys or

b) Ser65Thr.

59. The construct of claim 57 or 58, wherein the linker moiety comprises between 5 amino acids and 50 amino acids.

60. The construct of claim 57 or 58, wherein the donor moiety acceptor moiety and the linker moiety are fused in a single amino acid sequence.

61. The construct of claim 57 or 58, wherein the linker comprises a cleavage recognition site for trypsin, enterokinase, HIV-1 protease, prohormone convertase, interleukin-1b-converting enzyme, adenovirus endopeptidase, cytomegalovirus assemblin, leishmanolysin, b-Secretase for APP, thrombin, renin, angiotensin-converting enzyme, cathepsin D or a kininogenase.

62. A recombinant nucleic acid coding for expression of a tandem fluorescent protein construct, the construct, comprising a donor fluorescent protein moiety, an acceptor fluorescent protein moiety and a linker moiety that couples the donor and acceptor moieties and wherein the donor and acceptor moieties exhibit fluorescence resonance energy transfer ("FRET") when the donor moiety is excited by radiation, characterized in that the linker moiety comprises a protease cleavage recognition site, wherein cleavage of the linker by a protease results in a change in FRET between the donor and acceptor moieties,

and wherein the donor moiety comprises an *Aequorea* fluorescent protein (SEQ. ID. No. 2) comprising the amino acid substitutions,

a) Phe64Leu, Ser65Thr, Tyr66Trp, Asn146Ile, Met153Thr, Val163A and Asn212Lys, or
b) Ser65Gly, Val68Leu, Ser72Ala and Thr203Tyr, or
c) Tyr66His and Tyr145Phe, or
d) Tyr66Trp, Asn146Ile, Met153Thr, Val163Ala and Asn212Lys, or
e) Ser72Ala, Tyr145Phe and Thr203Ile, or
f) Ser65Thr, Ser72Ala, Asn149Lys, Met153Thr and Ile167Thr, and

the acceptor moiety comprises an *Aequorea* fluorescent protein (SEQ. ID. No. 2) comprising the amino acid substitutions,

a) Ser65Gly, Val68Leu, Ser72Ala and Thr203Tyr, or
b) Ser65Thr, Ser72Ala, Asn149Lys, Met153Thr and Ile167Thr.

63. A recombinant nucleic acid coding for expression of a tandem fluorescent protein construct, the construct comprising a donor fluorescent protein moiety, an acceptor fluorescent protein moiety and a linker moiety that couples the donor and acceptor moieties and wherein the donor and acceptor moieties exhibit fluorescence resonance energy transfer ("FRET") when the donor moiety is excited by radiation, characterized in that the linker moiety comprises a protease cleavage recognition site, wherein cleavage of the linker by a protease results in a change in FRET between the donor and acceptor moieties, and

wherein the donor moiety comprises an *Aequorea* fluorescent protein (SEQ. ID. No. 2) comprising the amino acid substitutions,

a) Tyr66His and Tyr145Phe, or

b) Tyr66Trp, Asn146Ile, Met153Thr, Val163Ala and Ans212Lys, and

the acceptor moiety comprises an *Aequorea* fluorescent protein (SEQ. ID. No. 2) comprising the amino acid substitutions,

a) Ser65Cys or

b) Ser65Thr.

64. The nucleic acid of claim 62 or 63, wherein the linker moiety comprises between 5 amino acids and 50 amino acids.

65. A host cell transfected with an expression vector comprising an expression control sequence operatively linked to a sequence coding for the expression of a tandem fluorescent protein construct, the construct comprising a donor fluorescent protein moiety, an acceptor fluorescent protein moiety and a linker moiety that couples the donor and acceptor moieties and wherein the donor and acceptor moieties exhibit fluorescence resonance energy transfer ("FRET") when the donor moiety is excited by radiation, characterized in that the linker moiety comprises a protease cleavage recognition site, wherein cleavage of the linker by a protease results in a change in FRET between the donor and acceptor moieties, and

wherein the donor moiety comprises an *Aequorea* fluorescent protein (SEQ. ID. No. 2) comprising the amino acid substitutions,

a) Phe64Leu, Ser65Thr, Tyr66Trp, Asn146Ile, Met153Thr, Val163A and Asn212Lys, or

b) Ser65Gly, Val68Leu, Ser72Ala and Thr203Tyr, or

c) Tyr66His and Tyr145Phe, or

d) Tyr66Trp, Asn146Ile, Met153Thr, Val163Ala and Asn212Lys, or

e) Ser72Ala, Tyr145Phe and Thr203Ile, or

f) Ser65Thr, Ser72Ala, Asn149Lys, Met153Thr and Ile167Thr, and

the acceptor moiety comprises an *Aequorea* fluorescent protein (SEQ. ID. No. 2) comprising the amino acid substitutions,

a) Ser65Gly, Val68Leu, Ser72Ala and Thr203Tyr, or

b) Ser65Thr, Ser72Ala, Asn149Lys, Met153Thr and Ile167Thr.

66. A host cell transfected with an expression vector comprising an expression control sequence operatively linked to a sequence coding for the expression of a tandem fluorescent protein construct, the construct comprising a donor fluorescent protein moiety, an acceptor fluorescent protein moiety and a linker moiety that couples the donor and acceptor moieties and wherein the donor and acceptor moieties exhibit fluorescence resonance energy transfer ("FRET") when the donor moiety is excited by radiation, characterized in that the linker moiety comprises a protease cleavage recognition site, wherein cleavage of the linker by a protease results in a change in FRET between the donor and acceptor moieties, and

wherein the donor moiety comprises an *Aequorea* fluorescent protein (SEQ. ID. No. 2) comprising the amino acid substitutions,

a) Tyr66His and Tyr145Phe, or

b) Tyr66Trp, Asn146Ile, Met153Thr, Val163Ala and Ans212Lys, and

the acceptor moiety comprises an *Aequorea* fluorescent protein (SEQ. ID. No. 2) comprising the amino acid substitutions,

a) Ser65Cys or

b) Ser65Thr.

67. The host cell of claim 65 or 66, further comprising a protease that is not naturally expressed by the host cell.

68. The host cell of claim 65 or 66, wherein the host cell is *E. coli.*

69. The host cell of claim 65 or 66, wherein the host cell is an eukaryotic cell.

70. The host cell of claim 65 or 66, wherein the host cell is a mammalian cell.

71. A method for measuring protease activity in a sample, comprising:

1) contacting the sample with the tandem fluorescent protein construct of claim 57 or 58 that comprises a linker moiety comprising a cleavage recognition site specific for the protease;

2) exciting the donor moiety by radiation; and

3) measuring fluorescence resonance energy transfer between the donor and acceptor moieties at a first time and a second time after addition of the tandem fluorescent protein construct whereby a decrease in fluorescence resonance energy transfer upon incubation of the sample with the tandem fluorescent protein construct indicates protease activity.

10057505.012502

72. A method of measuring protease activity in a cell, comprising the steps of:

1) providing a cell that expresses the tandem fluorescent protein construct, of claim 57 or 58 that comprises a linker moiety comprising a cleavage recognition site specific for the protease;

2) exciting the donor moiety by radiation; and

3) measuring the degree of fluorescence resonance energy transfer between the donor and acceptor moieties wherein cleavage of the construct by the protease results in less fluorescence resonance energy transfer which reflects protease activity.

73. The method of claim 72, wherein the step of providing a cell comprises; inducing a sudden increase in expression of the tandem fluorescent protein construct, and the step of measuring the degree of fluorescence resonance energy transfer comprises; determining the degree at a first and a second time after induction of tandem fluorescent protein construct expression and determining the difference between the first and second time, whereby less fluorescence resonance energy transfer reflects the presence of the protease.

74. A method for determining whether a compound alters the activity of a protease comprising the steps of:

contacting a sample containing a known amount of the protease with the compound and with the tandem fluorescent protein construct of claim 57 or 58;

exciting the donor moiety by radiation; and

determining the degree of fluorescence resonance energy transfer between the donor and acceptor moieties in the sample containing the compound, and comparing the degree of fluorescence resonance energy transfer between the donor and acceptor moieties in a sample not containing the compound, whereby a difference in the degree of fluorescence resonance energy transfer indicates that the compound alters the activity of the protease.

75. A method for determining whether a compound alters the activity of a protease in a cell, comprising the steps of:

1) providing first and second cells that express the tandem fluorescent protein construct of claims 57 or 58, wherein the linker moiety comprises a cleavage recognition amino acid sequence specific for the protease;

2) contacting the first cell with an amount of the compound;

3) contacting the second cell with a different amount of the compound, or a buffer control;

4) exciting the donor moiety in the first and second cell by radiation;

5) determining the degree of fluorescence resonance energy transfer in the first and second cells; and

6) comparing the degree of fluorescence resonance energy transfer in the first and second cells, whereby a difference in the degree of fluorescence resonance energy transfer indicates that the compound alters the activity of the protease.

76. A tandem fluorescent protein construct, comprising a donor fluorescent protein moiety, an acceptor fluorescent protein moiety and a linker moiety that couples the donor and acceptor moieties and wherein the donor and acceptor moieties exhibit fluorescence resonance energy transfer ("FRET") when the donor moiety is excited by radiation, characterized in that the linker moiety comprises a protease cleavage recognition site, wherein cleavage of the linker by a protease results in a change in FRET between the donor and acceptor moieties, and wherein the donor or acceptor moieties comprises either,

an *Aequorea* fluorescent protein (SEQ. ID. No. 2) comprising a mutation that reduces the hydrophobicity at positions A206, L221 or F223 and attenuates the intermolecular interactions between the donor or acceptor moieties,

or the donor moiety comprises an *Aequorea* fluorescent protein (SEQ. ID. No. 2) comprising the amino acid substitutions,

a) Phe64Leu, Ser65Thr, Tyr66Trp, Asn146Ile, Met153Thr, Val163A and Asn212Lys, or

b) Ser65Gly, Val68Leu, Ser72Ala and Thr203Tyr, or

c) Tyr66His and Tyr145Phe, or

d) Tyr66Trp, Asn146Ile, Met153Thr, Val163Ala and Asn212Lys, or

e) Ser72Ala, Tyr145Phe and Thr203Ile, or

f) Ser65Thr, Ser72Ala, Asn149Lys, Met153Thr and Ile167Thr,

or the acceptor moiety comprises an *Aequorea* fluorescent protein (SEQ. ID. No. 2) comprising the amino acid substitutions,

a) Ser65Gly, Val68Leu, Ser72Ala and Thr203Tyr, or

b) Ser65Thr, Ser72Ala, Asn149Lys, Met153Thr and Ile167Thr.

77. A recombinant nucleic acid coding for expression of a tandem fluorescent protein construct, the construct comprising construct comprising a donor fluorescent protein moiety, an acceptor fluorescent protein moiety and a linker moiety that couples the donor and acceptor moieties and wherein the donor and acceptor moieties exhibit fluorescence resonance energy transfer ("FRET") when the donor moiety is excited by radiation, characterized in that the linker moiety comprises a protease cleavage recognition site, wherein cleavage of the linker by a protease results in a change in FRET between the donor and acceptor moieties, and wherein the donor or acceptor moieties comprises either,

an *Aequorea* fluorescent protein (SEQ. ID. No. 2) comprising a mutation that reduces the hydrophobicity at positions A206, L221 or F223 and attenuates the intermolecular interactions between the donor or acceptor moieties,

or the donor moiety comprises an *Aequorea* fluorescent protein (SEQ. ID. No. 2) comprising the amino acid substitutions,

a) Phe64Leu, Ser65Thr, Tyr66Trp, Asn146Ile, Met153Thr, Val163A and Asn212Lys, or

b) Ser65Gly, Val68Leu, Ser72Ala and Thr203Tyr, or

c) Tyr66His and Tyr145Phe, or

d) Tyr66Trp, Asn146Ile, Met153Thr, Val163Ala and Asn212Lys, or

e) Ser72Ala, Tyr145Phe and Thr203Ile, or

f) Ser65Thr, Ser72Ala, Asn149Lys, Met153Thr and Ile167Thr,

or the acceptor moiety comprises an *Aequorea* fluorescent protein (SEQ. ID. No. 2) comprising the amino acid substitutions,

a) Ser65Gly, Val68Leu, Ser72Ala and Thr203Tyr, or

b) Ser65Thr, Ser72Ala, Asn149Lys, Met153Thr and Ile167Thr.

78. A host cell transfected with an expression vector comprising an expression control sequence operatively linked to a sequence coding for the expression of a tandem fluorescent protein construct, the construct comprising construct comprising a donor fluorescent protein moiety, an acceptor fluorescent protein moiety and a linker moiety that couples the donor and acceptor moieties and wherein the donor and acceptor moieties exhibit fluorescence resonance energy transfer ("FRET") when the donor moiety is excited by radiation, characterized in that the linker moiety comprises a protease cleavage recognition site, wherein cleavage of the linker by a protease results in a change in FRET between the donor and acceptor moieties, and wherein the donor or acceptor moieties comprises either,

an *Aequorea* fluorescent protein (SEQ. ID. No. 2) comprising a mutation that reduces the hydrophobicity at positions A206, L221 or F223 and attenuates the intermolecular interactions between the donor or acceptor moieties,

or the donor moiety comprises an *Aequorea* fluorescent protein (SEQ. ID. No. 2) comprising the amino acid substitutions,

a) Phe64Leu, Ser65Thr, Tyr66Trp, Asn146Ile, Met153Thr, Val163A and Asn212Lys, or

b) Ser65Gly, Val68Leu, Ser72Ala and Thr203Tyr, or

c) Tyr66His and Tyr145Phe, or

d) Tyr66Trp, Asn146Ile, Met153Thr, Val163Ala and Asn212Lys, or

e) Ser72Ala, Tyr145Phe and Thr203Ile, or

f) Ser65Thr, Ser72Ala, Asn149Lys, Met153Thr and Ile167Thr,

or the acceptor moiety comprises an *Aequorea* fluorescent protein (SEQ. ID. No. 2) comprising the amino acid substitutions,

a) Ser65Gly, Val68Leu, Ser72Ala and Thr203Tyr, or

b) Ser65Thr, Ser72Ala, Asn149Lys, Met153Thr and Ile167Thr.--

10057505.012502

II. REMARKS

Formal Drawings are submitted herewith to replace those originally filed with the parent application. With respect to the Formal Drawings, Figures 1B and 2 have been amended to correct typographical errors. Figure 1B was amended such that the nucleotide position indicated as "717" at the end of the sequence was changed to "716" (see original Figure 1), which is the correct number of nucleotides shown (see, also, SEQ ID NO:1). Figure 2 was amended to correct a misspelling of the term "acid." Marked versions of original Figure 1 and of Formal Drawing Figures 1A and 2 showing the amendments are attached as Exhibit A.

The specification has been amended to update the continuing information. As such, the amendment merely addresses a formality and does not add new matter.

Applicants have cancelled claims 1 to 57 and added new claims 58 to 78. The new claims do not introduce new matter and fully supported by the specification as originally filed. Specific support for the new claims is summarized in the Table below.

Claim Number	Support in Specification
57	Claims 1, 2, 4, 5, Table 1 page 16
58	Claims 1, 2, 3, 4, 5, 9, Table 1 page 16
59	Claim 7
60	Claim 6
61	Claim 10
62	Claims 16, 17,18, Table 1, page 16, pages 31 to 33
63	Claims 16, 17,18, Table 1, page 16, pages 31 to 33
64	Claim 7
65	Claim 22
66	Claim 22

Claim Number	Support in Specification
67	Claim 23
68	Claim 24
69	Claim 25
70	Claim 26
71	Claims 27 to 35, pages 35 to 40
72	Claims 36 to 39, pages 35 to 40
73	Claim 40
74	Claim 42, 44
75	Claim 45
76	Page 19, lines 20 to 33, Page 20 lines 5 to 7, Page 16 Table 1, claims 1, 2, 4, 5,
77	Page 19, lines 20 to 33, Page 20 lines 5 to 7, Page 16 Table 1, claims 16, 17,18
78	Page 19, lines 20 to 33, Page 20 lines 5 to 7, Page 16 Table 1, claims 22 to 24

10055656.020502

In view of the foregoing, Applicants respectfully submit that the claims are ready for examination and are in condition for allowance. Please apply any charges not covered, or any credits, to Deposit Account 50-1355. The Examiner is invited to contact Applicants' undersigned representative if there are any questions relating to this application.

Respectfully submitted,

Date: January 25, 2002

Lisa A. Haile, J.D., Ph.D.
Registration No. 38,347
Telephone: (858) 677-1456
Facsimile: (858) 677-1465

USPTO Customer Number 28213
GRAY CARY WARE & FREIDENRICH LLP
4365 Executive Drive, Suite 1100
San Diego, CA 92121-2133

(xi) SEQUENCE DESCRIPTION:

SEQ ID NO:1:

```
ATG AGT AAA GGA GAA GAA CTT TTC ACT GGA GTT GTC CCA ATT CTT GTT      48
```

SEQ ID NO:2:

```
Met Ser Lys Gly Glu Glu Leu Phe Thr Gly Val Val Pro Ile Leu Val
 1               5                  10                  15

GAA TTA GAT GGT GAT GTT AAT GGG CAC AAA TTT TCT GTC AGT GGA GAG      96
Glu Leu Asp Gly Asp Val Asn Gly His Lys Phe Ser Val Ser Gly Glu
            20                  25                  30

GGT GAA GGT GAT GCA ACA TAC GGA AAA CTT ACC CTT AAA TTT ATT TGC     144
Gly Glu Gly Asp Ala Thr Tyr Gly Lys Leu Thr Leu Lys Phe Ile Cys
        35                  40                  45

ACT ACT GGA AAA CTA CCT GTT CCA TGG CCA ACA CTT GTC ACT ACT TTC     192

Thr Thr Gly Lys Leu Pro Val Pro Trp Pro Thr Leu Val Thr Thr Phe
    50                  55                  60

TCT TAT GGT GTT CAA TGC TTT TCA AGA TAC CCA GAT CAT ATG AAA CGG     240
Ser Tyr Gly Val Gln Cys Phe Ser Arg Tyr Pro Asp His Met Lys Arg
65                  70                  75                  80

CAT GAC TTT TTC AAG AGT GCC ATG CCC GAA GGT TAT GTA CAG GAA AGA     288
His Asp Phe Phe Lys Ser Ala Met Pro Glu Gly Tyr Val Gln Glu Arg
                85                  90                  95

ACT ATA TTT TTC AAA GAT GAC GGG AAC TAC AAG ACA CGT GCT GAA GTC     336
Thr Ile Phe Phe Lys Asp Asp Gly Asn Tyr Lys Thr Arg Ala Glu Val
            100                 105                 110

AAG TTT GAA GGT GAT ACC CTT GTT AAT AGA ATC GAG TTA AAA GGT ATT     384
Lys Phe Glu Gly Asp Thr Leu Val Asn Arg Ile Glu Leu Lys Gly Ile
        115                 120                 125

GAT TTT AAA GAA GAT GGA AAC ATT CTT GGA CAC AAA TTG GAA TAC AAC     432
Asp Phe Lys Glu Asp Gly Asn Ile Leu Gly His Lys Leu Glu Tyr Asn
    130                 135                 140

TAT AAC TCA CAC AAT GTA TAC ATC ATG GCA GAC AAA CAA AAG AAT GGA     480
Tyr Asn Ser His Asn Val Tyr Ile Met Ala Asp Lys Gln Lys Asn Gly
145                 150                 155                 160

ATC AAA GTT AAC TTC AAA ATT AGA CAC AAC ATT GAA GAT GGA AGC GTT     528
Ile Lys Val Asn Phe Lys Ile Arg His Asn Ile Glu Asp Gly Ser Val
                165                 170                 175

CAA CTA GCA GAC CAT TAT CAA CAA AAT ACT CCA ATT GGC GAT GGC CCT     576
Gln Leu Ala Asp His Tyr Gln Gln Asn Thr Pro Ile Gly Asp Gly Pro
            180                 185                 190

GTC CTT TTA CCA GAC AAC CAT TAC CTG TCC ACA CAA TCT GCC CTT TCG     624
Val Leu Leu Pro Asp Asn His Tyr Leu Ser Thr Gln Ser Ala Leu Ser
        195                 200                 205

AAA GAT CCC AAC GAA AAG AGA GAC CAC ATG GTC CTT CTT GAG TTT GTA     672
Lys Asp Pro Asn Glu Lys Arg Asp His Met Val Leu Leu Glu Phe Val
    210                 215                 220

ACA GCT GCT GGG ATT ACA CAT GGC ATG GAT GAA CTA TAC AAA TA          717
Thr Ala Ala Gly Ile Thr His Gly Met Asp Glu Leu Tyr Lys
225                 230                 235
```

716

FIGURE 1

```
GAT TTT AAA GAA GAT GGA AAC ATT CTT GGA CAC AAA TTG GAA TAC AAC      432
Asp Phe Lys Glu Asp Gly Asn Ile Leu Gly His Lys Leu Glu Tyr Asn
    130                 135                 140

TAT AAC TCA CAC AAT GTA TAC ATC ATG GCA GAC AAA CAA AAG AAT GGA      480
Tyr Asn Ser His Asn Val Tyr Ile Met Ala Asp Lys Gln Lys Asn Gly
145                 150                 155                 160

ATC AAA GTT AAC TTC AAA ATT AGA CAC AAC ATT GAA GAT GGA AGC GTT      528
Ile Lys Val Asn Phe Lys Ile Arg His Asn Ile Glu Asp Gly Ser Val
                165                 170                 175

CAA CTA GCA GAC CAT TAT CAA CAA AAT ACT CCA ATT GGC GAT GGC CCT      576
Gln Leu Ala Asp His Tyr Gln Gln Asn Thr Pro Ile Gly Asp Gly Pro
            180                 185                 190

GTC CTT TTA CCA GAC AAC CAT TAC CTG TCC ACA CAA TCT GCC CTT TCG      526
Val Leu Leu Pro Asp Asn His Tyr Leu Ser Thr Gln Ser Ala Leu Ser
        195                 200                 205

AAA GAT CCC AAC GAA AAG AGA GAC CAC ATG GTC CTT CTT GAG TTT GTA      672
Lys Asp Pro Asn Glu Lys Arg Asp His Met Val Leu Leu Glu Phe Val
    210                 215                 220

ACA GCT GCT GGG ATT ACA CAT GGC ATG GAT GAA CTA TAC AAA TA           717
Thr Ala Ala Gly Ile Thr His Gly Met Asp Glu Leu Tyr Lys
225                 230                 235                          716
```

FIG. 1B

2/10

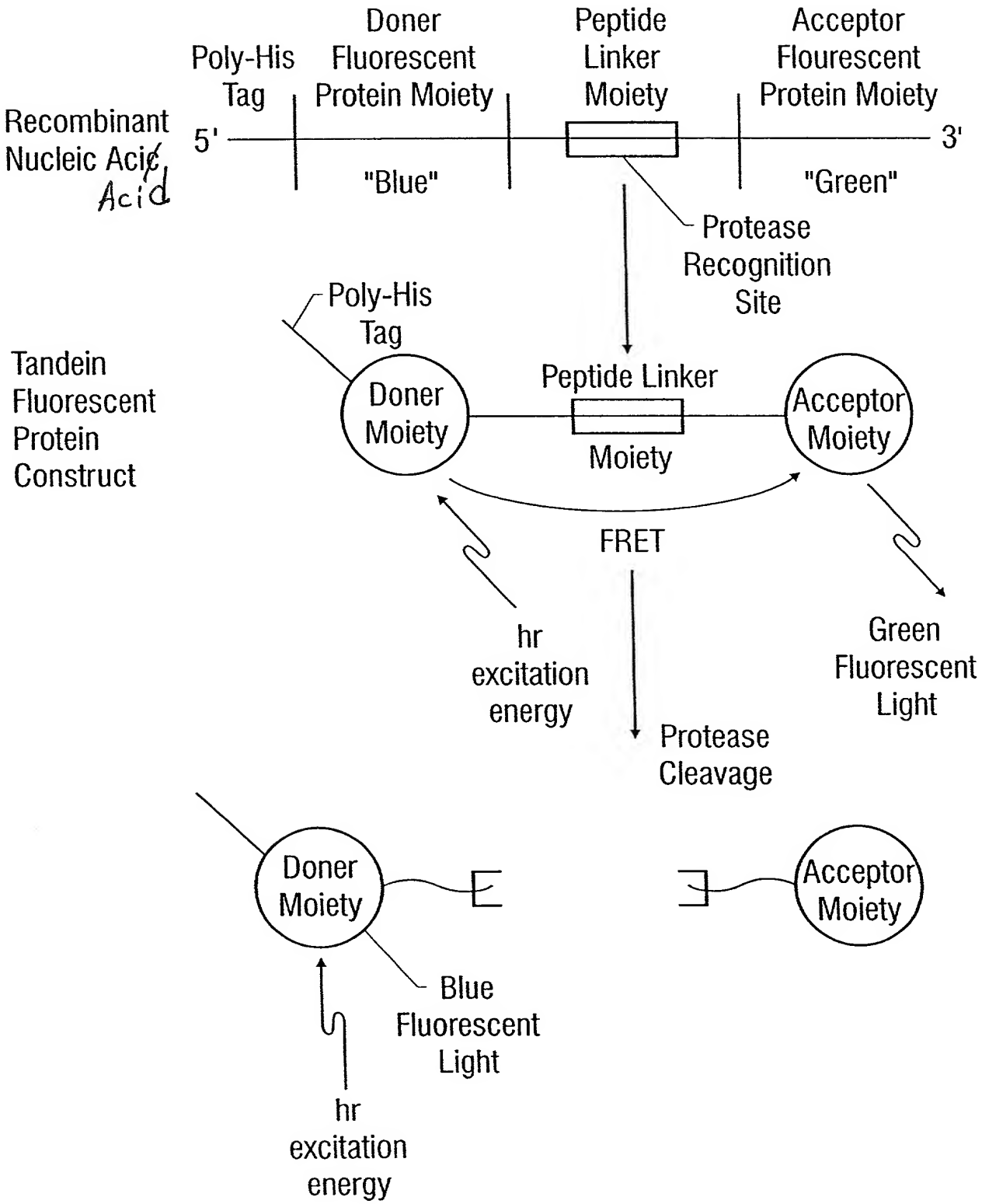
Recombinant Nucleic Acid
Poly-His Tag
Doner Fluorescent Protein Moiety
Peptide Linker Moiety
Acceptor Flourescent Protein Moiety
5'
3'
"Blue"
"Green"
Protease Recognition Site
Tandein Fluorescent Protein Construct
Poly-His Tag
Doner Moiety
Peptide Linker Moiety
Acceptor Moiety
FRET
hr excitation energy
Green Fluorescent Light
Protease Cleavage
Doner Moiety
Acceptor Moiety
Blue Fluorescent Light
hr excitation energy

FIG. 2